REMARKS

As an initial matter, the outstanding Office action did not account for the Preliminary Amendment submitted as part of the Rule 53(d) continuing application, filed December 15, 1998. The transmittal papers for the continuing application included the amendment to: "Cancel claims 46-68 and 70-106" (emphasis in original). According to the outstanding Office action, claims 39-106 were "pending in the application" and claims 85-101 were "rejected." Accordingly, the outstanding Office action did not reflect examination of the claims that remain pending in the application. Applicants respectfully submit that the next Office action issued should be a non-final action.

In conjunction with the claims originally submitted for examination, applicants had constructively elected to prosecute the claims of Group I, pursuant to the restriction requirement, and species II, pursuant to the requirement for election of species, both made in paper #10 in the parent application. As to the claims presented, hereby (as discussed, below), applicants maintain the constructive election to prosecute the claims of Group I and the constructive election of species II, with traverse. Traversal of the restriction requirement and election-of-species requirement is for the same reasons as set forth in applicants response filed on or about June 16, 1997, incorporated herein by reference.

Applicants wish to thank Examiner P. Achutamurthy for the courteous assistance rendered to applicants' representative during an interview at the U.S. Patent and Trademark Office (PTO) on April 1, 1999. The preliminary amendment not being taken into account was discussed with the examiner during the interview.

The claims presented for examination are claims 107-147. Support for new claim 107 is found in original claims 1 and 14 (for the recited feature "measuring volume is arranged at a distance of $\leq$ 1000 μm from the laser focusing optics," also described on page 33, paragraph 12, of the specification).

Claims 108 and 109 correspond to preferred embodiments disclosed in original claim 1.

Claims 110 and 111 correspond to original claims 2 and 4.

Claim 112 corresponds to original claim 5.

Claim 113 corresponds to original claim 6. Changes were incorporated to better reflect the applicants' intention and to overcome the examiner's rejections for the use of the phrase "and/or," which in the examiner's opinion renders the claim vague and indefinite.

Claims 114-119 correspond to original claims 7-11.

Claims 120 and 121 correspond to preferred embodiments of original claim 11.

Claims 122 and 123 correspond to original claims 12 and 13.

Claim 124 corresponds to original claim 14.

Claims 125-127 correspond to preferred embodiments of former claim 15.

Claim 128 corresponds to original claim 16.

Claim 129 corresponds to original claim 17. Preferred embodiments recited in original claim 17 were canceled to overcome the examiner's rejection under §112.

Claims 130 and 131 correspond to original claim 18. The claim has been split to overcome the examiner's rejection with respect to 35 U.S.C. § 112.

Claim 132 corresponds to original claim 19.

Claims 133 and 134 correspond to original claims 20 and 21 except for omissions of preferred embodiments to overcome the examiner's rejections.

Claim 135-142 and 144-147 correspond to original claims 23-37. Only minor changes regarding indefinite terms like "small," "short" and wordings like "preferably" and "especially" were made to overcome the examiner's rejection.

Dependent claim 143 corresponds to preferred embodiments of former claim 33.

Claims 1-2, 4-10, 12-16, 22 and 28-38 were rejected under 35 USC 112, first paragraph, for allegedly lacking enablement. Reconsideration is respectfully requested.

The examiner stated that one skilled in the art is not able to identify a chemical species by the parameters recited in the instant claims. According to the examiner, it is not disclosed, for example, how to identify divergent species of similar size and rotational diffusion coefficient. In addition to this, the examiner refers to claim 4, which recited that coordinates of the measuring compartment, itself, change with time and defined diffusion time for a luminescent complex. The examiner concluded that one of ordinary skill in the art would not predict that motion of a compartment will correlate with diffusion of a complex contained therein.

Applicants respectfully disagree with the examiner's analysis and conclusion with respect to the §112, first paragraph, rejection.

The claims were rejected because it is allegedly not possible to identify molecules via FCS. In the Examiner's opinion the word "identify" means to find out the exact chemical constitution of a molecule. Applicants have amended the claims to recite "assay," rather than *identify*. To illustrate the use of FCS, imagine, e.g., the following non-limiting example in a drug discovery process (see the figure attached, hereto, as Appendix I). One might have an assay comprising a receptor

having specific binding properties for a luminescently-labeled ligand. A fraction of the ligand will bind to the receptor; whereas, another fraction of the ligand will remain unbound. In a FCS analysis this can be seen by the fact that the first fraction has a slow translational diffusion; whereas, the second fraction is characterized by a fast translational diffusion. When a potentially active compound is added, an equilibrium shift indicates interaction of the compound with the specific binding receptor.

In regard to the rejection of original claim 4, with all due respect, the examiner appears to misinterpret the meaning of the claim's wording. In FCS, random fluctuations of the intensity of individual molecules excited to fluorescence by a stationary light source provide information on important molecule properties, such as translational diffusion. An objective of the presently claimed invention is to observe the intensity fluctuations of molecules excited to fluorescence in a tiny volume element. These variations are due to fluctuations in the number of molecules when entering or leaving the measuring volume. As disclosed on pages 37 and 38 of the specification, the method according to the presently claimed invention can also be performed involving molecules that do not fluctuate or fluctuate very slowly. If the molecules to be assayed as well as the light source are stationary, then one would measure a constant nonfluctuating intensity. According to the presently claimed invention, one can intentionally change the coordinates of

the measuring volume with respect to the coordinates of the sample by moving the light source or by moving the sample itself. As a consequence one would measure intensity fluctuations. These fluctuations are created by molecules entering and leaving the measuring volume due to the coordinate changes. In the case of fixed or very slowly diffusing molecules, these coordinate changes are the major source for the intensity fluctuations and do not reflect the innate properties of the molecules - they just reflect the velocity of the coordinate change. In the case of freely diffusing molecules the fluctuations caused by the coordinate change and the free diffusion are superimposed to a joint correlation curve. The correlation time is an apparent diffusion time since the effect of the coordinate change has to be subtracted in order to determine the real diffusion time.

Claims were rejected under 35 USC 112, second paragraph, for allegedly being indefinite due allegedly "omitted steps." Reconsideration is requested.

To overcome these rejections applicants refer to the new set of claims added. It is respectfully submitted that the claims, as amended hereby, satisfy the standards for definite language set forth in § 112, second paragraph. Further, applicants respectfully remark that "it is not necessary that a claim recite each and every element needed for the practical utilization of the claimed subject matter." *Carl Zeiss Stiftung v. Renishaw PLC,* 20 USPQ2d 1094, 1101 (Fed. Cir. 1991). Explaining how the invention is to be practiced is the function of the specification;

the function of the claims is to define the legal limits of the invention. *In re Roberts*, 176 USPQ 313, 314 ((CCPA 1973).

Claims 1-2, 4-10, 12-16, 22 and 28-38 were rejected under 35 USC 112, first paragraph, for allegedly lacking enablement. Reconsideration is respectfully requested.

Claims 1-2, 5-8, 10, 15, 28-30 and 38 were rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Thompson et al. *Biophys. J.* (1983) 43:103-114 (D1), or Soracher et al. *Biochimica et Biophysica Acta* (1980) 610:28-46 (D2), or Kask et al. *Eur. Biophys. J.* (1985) 12:163-166 (D3), or Rigler et al. *Fluorescence Spectroscopy* (Wolfbeis, editor) Springer Verlag (1992) 13-24 (D4), or Meyer et al. *Biophys. J.* (1988) 54:983-993 (D5). Reconsideration is respectfully requested.

To support a rejection under § 102 of the statute, each and every claim limitation, as arranged in the claim, must be found in a single prior art reference. *Jamesbury Corp. v. Litton Industrial Products, Inc.*, 225 USPQ 253 (Fed. Cir. 1985). The absence from a prior art reference of a single claim limitation negates anticipation. *Jamesbury Corp.*

Claim 107 contains the limitation that the measuring volume is arranged at a distance of less than or equal to ("$\leq$") 1000 μm from a laser focusing optic. This

express limitation of the so-called working distance is not found in any of the cited references.

Applicants respectfully direct the examiner's attention to the "Table" attached, hereto, as Appendix II. The Table displays the main properties of the inventive method in comparison to the methods disclosed in publications D1-D5.

This table clearly shows that only D5 describes a specific working distance. Applicants point to the fact that this distance of 14000 μm is far out of the range which is taught by the invention. Since each and any limitation of claim 107 is not found in any one of the cited references, this claim and all dependent claims are not anticipated. Therefore, applicants respectfully request that the rejections under 35 U.S.C. § 102 be withdrawn.

Claims 1-2, 5-8, 9-10, 12-16, 22, 28-30 and 31-38 were rejected under 35 U.S.C. § 103 as being allegedly unpatentable over Thompson et al., Soracher et al., Kask et al, Rigler, et al, or Meyer et al. – references D1-D5, cited in the statement of rejection under §102. Reconsideration is respectfully requested.

The claimed subject matter and the prior art are, allegedly, such that the subject matter as a whole, allegedly, would have been obvious at the time the invention was made to a person having ordinary skill in the art. Applicants respectfully traverse the rejection.

The limitation in the instant claims to a working distance, i.e., whereby, "said measuring volume is arranged *at a distance of* $\leq$ *1000 µm* from a laser focusing optic" (emphasis added), is not described in any of the references relied upon in the statement of rejection, as explained above with respect to the rejection under §102. Moreover, there is nothing in any of the art relied upon, taken alone or in combination, that would have suggested the *desirability* of such a working distance. Therefore, there is no obviousness under §103. As explained in *In re Fritch*, 23 USPQ2d 1780, 1783-84 (Fed. Cir. 1992):

> The mere fact that the prior art may be modified in the manner suggested by the Examiner does not make the modification obvious unless the prior art suggested the *desirability* of the modification. {*Emphasis, added.*]

More specifically, applicants refer, again, to the Table in Appendix II.

D1 deals with the experimental application of total internal reflection with fluorescence correlation spectroscopy (TIR/FCS) for measuring the binding and unbinding rates and surface diffusion coefficient of fluorescent-labeled solute molecules. These molecules are in chemical and thermodynamic equilibrium between a solution and a surface to which the reversibly adsorb. A laser beam totally internally reflects at the surface-solution interface, forming an electromagnetic field, called the "evanescent wave" in a very thin layer of solution immediately adjacent to the surface. Therefore, only those fluorescent solute molecules that are adsorbed to the surface are selectively excited by the

evanescent wave. As individual molecules bind and unbind within the observation area, or diffuse along the surface through it, the measured fluorescence fluctuates. The autocorrelation function depends on the rates of association, dissociation, and surface diffusion of the molecules. As an example the binding of Rhodamine labeled DNP-antibodies to a dinitrophenol (DNP) coated surface has been examined. In contrast to the method according to the present invention, the TIR/FCS method disclosed in D1 is only suitable to measure adsorption and desorption kinetic rates of molecules binding to a surface element. D1 does not disclose a specific working distance nor are the measuring times smaller than 500 ms. In the study of surface concentration fluctuations in R-IgG on BSA-glass, these fluorescence fluctuations are autocorrelated with reasonable statistical accuracy in 5 to 45 minutes.

The publication D2 discloses the application of FCS to characterized calf thymus DNA, SV 40 DNA and calf thymus nucleohistone particles by binding of ethidium bromide. Furthermore, small regions of individual nuclei are examined. The main goal of Soracher et al. was to decide by the amount of ethidium bromide bound to the chromatin of the nucleus if the cell is stimulated (growing) or resting. Moreover, this publication lacks any disclosure about the working distance and the measuring times.

D3 deals with the possibilities for the use of fluorescence correlation spectroscopy in the nanosecond time range. The here described experiment is

based on a cw argon ion laser, a microfluorimeter, two photon detectors and a time-to-analog converting system. The experiments were carried out on Rhodamine 6G and pyronine G for which a photon anticorrelation component decaying with a time constant close to the excited state life-time was observed. The duration of every experiment was 9 hours which is far out of the range of measuring times ≤ 500 ma taught by the present invention. Kask et al. do not disclose a value for the working distance in their paper.

The publication D4 by Rigler et al. deals with interactions and kinetics of single molecules observed by FCS. Contrary to relaxation experiments, the system's thermodynamic fluctuations themselves are observed. Random fluctuations of the intensity of individual molecules excited to fluorescence by a laser light source and the corresponding signal are recorded. The signals are analyzed by calculating the correlation function of the fluorescence intensity fluctuations and derivative thereof the translational diffusion, the rotational motion and its coupling to the decay of the excited states. Rigler et al. do not disclose any working distance in their paper.

In D5 particle counting by fluorescence correlation spectroscopy is described. Meyer et al. disclose a method for simultaneous determination of molar weights and lateral diffusion constants of particles in three- and two-dimensional systems. Spontaneous concentration fluctuations in space and time are analyzed by

monitoring fluctuation in the fluorescence from fluorescein-labeled molecules, excited by a rotating laser spot. This so-called fluorescence particle counting (FPC) technique allows to directly count the number of independent particles in a defined volume. To perform the measurements the objective is placed at a distance of 14000 μm in respect to the sample. Measuring times of minutes are disclosed.

To summarize, the method disclosed in the above-mentioned papers do not anticipate or render obvious the inventive method. None of these papers discloses a method comparable to the inventive method. None of these papers discloses a working distance of $\leq$ 1000 μm.

D5 discloses a working distance of 14 mm. Therefore, one skilled in the art would accept 14 mm as a convenient wording distance. Contrary to the assumption that only such large working distances are suited for FCS, a distance of $\leq$ 1000 μm between a measuring volume and a laser focusing optics surprisingly has the following advantages:

1. Changes in the refraction number of the sample solution interfere negatively with the experimental method of assaying molecules. These changes cause changes in the geometry of the measuring volume, so that the translational diffusion time of the molecules apparently varies. Correction for such changes in refraction number by mathematical models or through internal standards is only possible, if the caused enlargement of the measuring volume is uniform in all directions. In case the measuring volume is distorted, correction by

known mathematical models is not sufficient. In addition, known correction methods are not applicable if the geometry of the measuring volume changes with time. Applicants have found out that the amplitudes of these changes increase in an unacceptable manner if the working distance is chosen too large. Thermostatization of samples during measurements causes convections which in turn cause local changes in the viscosity and the refraction number of the sample. Under such conditions, the analysis of FCS data would become uncertain and the reproducibility of the measurements would be low, because the mathematical model of correlation analysis is not suited to correct adequately for these changes. Using a distance of the measuring volume from the laser focusing optics $\leq$ 1000 μm leads to an improved reproducibility of the measurements.

2. Excitation radiation as well as the emitted fluorescence light pass the solution between the measuring volume and the laser focusing optics. This often leads to light scattering effects. This light scattering also causes an enlargement and distortion of the measuring volume, leading to the above-mentioned problems and effects on the reproducibility and correctness of the measurements. The signal-to-noise ratio is worsened. All these effects can be minimized by the use of a working distance of $\leq$ 1000 μm according to the present invention.
3. Absorption of the emitted fluorescence light occurring in the emission light path reduces the count rate per molecule. Applicants found that - contrary to the expectations and the knowledge of one or ordinary skill in the art - these effect influences the signal-to-noise ratio to a linear or higher extent. The disclosed working distance also minimizes these effects and increases the signal-to-noise ratio.

4. The method according to the present invention is very useful in the analysis of small amounts of biological samples, e.g., body solutions. The analysis of such samples causes a lot of problems because of light scattering effects caused by molecule complexes, microparticles or cells and by absorption of emitted light or by inhomogenities in the refraction number. These effects can also be minimized by using a working distance of $\leq$ 1000 μm according to the present invention.

As mentioned, above, the modification of the prior art needed to effect the claimed invention cannot have been "obvious unless the prior art suggested the desirability of the modification." *Fritch* at 1783-84. In the present situation, the statement of rejection contains no indication of either how the prior art suggested the "desirability" of the "distance between measuring volume and a laser focusing optics" recited in the instant claims, or where among the teachings of the cited references such a desirability is to be found. Where "the PTO asserts that there is an explicit or implicit teaching or suggestion in the prior art, it must indicate where such a teaching or suggestion appears in the reference." *In re Rijckaert,* 28 USPQ2d 1955, 1957 (Fed. Cir. 1993). The PTO may not "resort to speculation, unfounded assumptions or hindsight reconstruction to supply deficiencies in the factual basis." *In re Warner*, 154 USPQ 173, 178 ((CCPA 1967).

Applicants note the argument in the statement of rejection that missing limitations are deemed by the examiner to be obvious matter of *optimizing* parameters. With all due respect, mere argument that parameters recited in the

claims result from "optimizing" variables found in the prior art is an improper basis to reject a claim for obviousness. *In re Antonie*, 195 USPQ 6, 8 ((CCPA 1977). The standard for rejection under §103 is obviousness, not *optimizing*.

Since the prior art cited in the statements of rejection neither teach nor suggest a procedure assaying molecules in a sample by laser excited FCS, wherein the measuring volume is arranged at a distance $\leq$ 1000 μm from the laser focusing optic, in the manner presently claimed, the instant claims would not have been obvious over the prior art.

Favorable action is requested.

Respectfully submitted,

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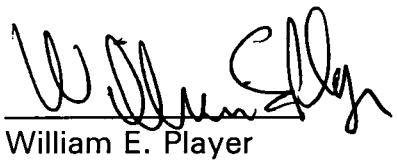

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